(54) Title: A METHOD AND APPARATUS FOR PREDICTING THE PRESENCE OF CONGENITAL AND ACQUIRED IMBALANCES AND THERAPEUTIC CONDITIONS
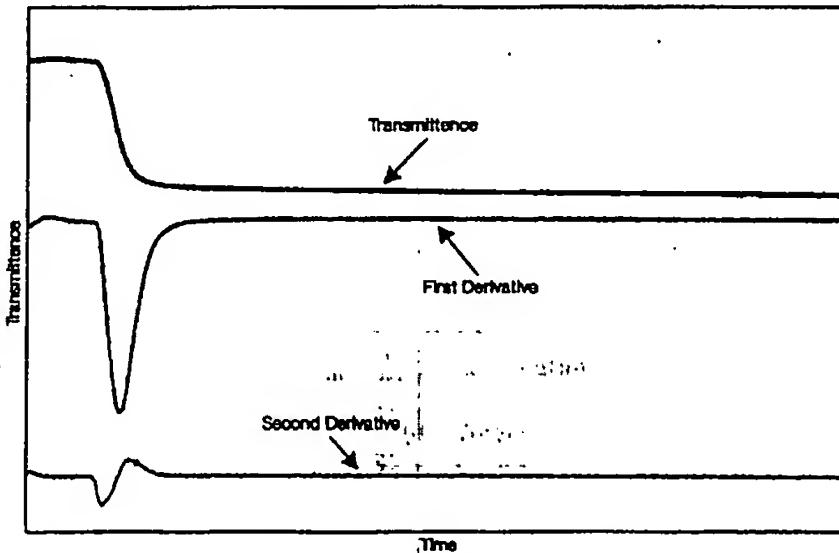
Normal APTT Optical Profile with First and Second Derivative

(57) Abstract

A method and apparatus are disclosed for predicting the presence of at least one congenital or acquired imbalance or therapeutic condition associated with thrombosis/hemostasis from at least one time-dependent measurement profile, (Figs. 3-6). At least one time-dependent measurement on an unknown sample is performed and a respective property of said sample is measured over time so as to derive a time-dependent measurement profile, (Figs. 3-6). A set of a plurality of predictor variables are defined which sufficiently define the data of the time-dependent measurement profile, (Fig. 13). A model is then derived that represents the relationship between the congenital or acquired imbalance or therapeutic condition, and the set of predictor variables. Subsequently, the model is utilized to predict the existence of the congenital or acquired imbalance or therapeutic condition in the unknown sample.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgystan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic	SD	Sudan
CF	Central African Republic		of Korea	SE	Sweden
CG	Congo	KR	Republic of Korea	SG	Singapore
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LR	Liberia	SZ	Swaziland
CS	Czechoslovakia	LT	Lithuania	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	LV	Latvia	TJ	Tajikistan
DK	Denmark	MC	Monaco	TT	Trinidad and Tobago
EE	Estonia	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	UG	Uganda
FI	Finland	ML	Mali	US	United States of America
FR	France	MN	Mongolia	UZ	Uzbekistan
GA	Gabon	MR	Mauritania	VN	Viet Nam

-1-

A Method and Apparatus for Predicting
The Presence of Congenital and Acquired
Imbalances and Therapeutic Conditions

5

BACKGROUND OF THE INVENTION

10

This application is a continuation-in-part of U.S. patent application 08/389,986 to Fischer et al. filed February 14, 1995, the subject matter of which is incorporated herein by reference. This application is also related to the following publications, the subject matter of each also being incorporated herein by reference:

1.   B. Pohl, C. Beringer, M. Bomhard, F. Keller, The quick machine - a mathematical model for the extrinsic activation of coagulation, *Haemostasis*, **24**, 325-337 (1994).

2.   J. Brandt, D. Triplett, W. Rock, E. Bovill, C. Arkin, Effect of lupus anticoagulants on the activated partial thromboplastin time, *Arch Pathol Lab Med*, **115**, 109-14 (1991).

3.   I. Talstad, Which coagulation factors interfere with the one-stage prothrombin time?, *Haemostasis*, **23**, 19-25 (1993).

4.   P. Baumann, T. Jurgensen, C. Heuck, Computerized analysis of the in vitro activation of the plasmatic clotting system, *Haemostasis*, **19**, 309-321 (1989).

5.   C. Heuck, P. Baumann, Kinetic analysis of the clotting system in the presence of heparin and depolymerized heparin, *Haemostasis*, **21**, 10-18 (1991).

6.   M. Astion and P. Wilding, The application of backpropagation neural networks to problems in pathology and laboratory medicine, *Arch Pathol Lab Med*, **116**, 995-1001 (1992).

-2-

7. M. Astion, M. Wener, R. Thomas, G. Hunder, and D. Bloch, Overtraining in neural networks that interpret clinical data, *Clinical Chemistry*, **39**, 1998-2004 (1993).

8. J. Furlong, M. Dupuy, and J. Heinsimer, Neural network analysis of serial cardiac enzyme data, *A.J.C.P.*, **96**, 134-141 (1991).

9. W. Dassen, R. Mulleneers, J. Smeets, K. den Dulk, F. Cruz, P. Brugada, and H. Wellens, Self-learning neural networks in electrocardiography, *J. Electrocardiol*, **23**, 200-202 (1990).

10. E. Baum and D. Haussler, What size net gives valid generalization? *Advances in Neural Information Processing Systems*, Morgan Kauffman Publishers, San Mateo, CA, 81-90 (1989).

11. A. Blum, *Neural Networks in C++*, John Wiley & Sons, New York, (1992).

12. S. Haykin, *Neural Networks A Comprehensive Foundation*, Macmillan College Publishing Company, New York, (1994).

13. J. Swets, Measuring the accuracy of diagnostic systems, *Science*, **240**, 1285-1293 (1988).

14. M. Zweig and G. Campbell, Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine, *Clinical Chemistry*, **39**, 561-577 (1993).

15. D. Bluestein, L. Archer, The sensitivity, specificity and predictive value of diagnostic information: a guide for clinicians, *Nurse Practitioner*, 16, 39-45 (1991).

16. C. Schweiger, G. Soeregi, S. Spitzauer, G. Maenner, and A. Pohl, Evaluation of laboratory data by conventional statistics and by three types of neural networks, *Clinical Chemistry*, **39**, 1966-1971 (1993).

-3-

Blood clots are the end product of a complex
chain reaction where proteins form an enzyme cascade
acting as a biologic amplification system. This
system enables relatively few molecules of initiator
5   products to induce sequential activation of a series
of inactive proteins, known as factors, culminating in
the production of the fibrin clot. Mathematical
models of the kinetics of the cascade's pathways have
been previously proposed.

10   In [1], a dynamic model of the extrinsic
coagulation cascade was described where data were
collected for 20 samples using quick percent,
activated partial thromboplastin time (APTT), thrombin
time (TT), fibrinogen, factor(F) II, FV, FVII, FX,
15   anti-thrombin III (ATIII), and factor degradation
product (FDP) assays. These data were used as input
to the model and the predictive output compared to
actual recovered prothrombin time (PT) screening assay
results. The model accurately predicted the PT result
20   in only 11 of 20 cases. These coagulation cascade
models demonstrate: (1) the complexity of the clot
formation process, and (2) the difficulty in
associating PT clot times alone with specific
conditions.

25   Thrombosis and hemostasis testing is the in vitro
study of the ability of blood to form clots and to
break clots in vivo. Coagulation (hemostasis) assays
began as manual methods where clot formation was
observed in a test tube either by tilting the tube or
30   removing fibrin strands by a wire loop. The goal was
to determine if a patient's blood sample would clot
after certain materials were added. It was later
determined that the amount of time from initiation of
the reaction to the point of clot formation in vitro
35   is related to congenital disorders, acquired
disorders, and therapeutic monitoring. In order to

-4-

remove the inherent variability associated with the
subjective endpoint determinations of manual
techniques, instrumentation has been developed to
measure *clot time*, based on (1) electromechanical
5   properties, (2) clot elasticity, (3) light scattering,
(4) fibrin adhesion, and (5) impedance. For light
scattering methods, data is gathered that represents
the transmission of light through the specimen as a
function of time (an *optical time-dependent*
10   *measurement profile*).

Two assays, the PT and APTT, are widely used to
screen for abnormalities in the coagulation system,
although several other screening assays can be used,
e.g. protein C, fibrinogen, protein S and/or thrombin
15   time. If screening assays show an abnormal result,
one or several additional tests are needed to isolate
the exact source of the abnormality. The PT and APTT
assays rely primarily upon measurement of time
required for clot time, although some variations of
20   the PT also use the amplitude of the change in optical
signal in estimating fibrinogen concentration.

Blood coagulation is affected by administration
of drugs, in addition to the vast array of internal
factors and proteins that normally influence clot
25   formation. For example, heparin is a widely-used
therapeutic drug that is used to prevent thrombosis
following surgery or under other conditions, or is
used to combat existing thrombosis. The
administration of heparin is typically monitored using
30   the APTT assay, which gives a prolonged clot time in
the presence of heparin. Clot times for PT assays are
affected to a much smaller degree. Since a number of
other plasma abnormalities may also cause prolonged
APTT results, the ability to discriminate between
35   these effectors from screening assay results may be
clinically significant.

-5-

Using a sigmoidal curve fit to a profile,
Baumann, et al [4] showed that a ratio of two
coefficients was unique for a select group of blood
factor deficiencies when fibrinogen was artificially

5   maintained by addition of exogenous fibrinogen to a
fixed concentration, and that same ratio also
correlates heparin to FII deficiency and FXa
deficiencies. However, the requirement for
artificially fixed fibrinogen makes this approach

10   inappropriate for analysis of clinical specimens. The
present invention makes it possible to predict a
congenital or acquired imbalance or therapeutic
condition for clinical samples from a time-dependent
measurement profile without artificial manipulation of

15   samples.
The present invention was conceived of and
developed for predicting the presence of congenital or
acquired imbalances or therapeutic conditions of an
unknown sample based on one or more time-dependent

20   measurement profiles, such as optical time-dependent
measurement profiles, where a set of predictor
variables are provided which define characteristics of
profile, and where in turn a model is derived that
represents the relationship between a congenital or

25   acquired imbalance or therapeutic condition and the
set of predictor variables (so as to, in turn, utilize
this model to predict the existence of the congenital
or acquired imbalance or therapeutic condition in the
unknown sample).

30

SUMMARY OF THE INVENTION

The present invention is directed to a method and
apparatus for predicting the presence of at least one
congenital or acquired imbalance or therapeutic

35   condition from at least one time-dependent measurement

-6-

profile. The method and apparatus include a)
performing at least one assay on an unknown sample and
measuring a respective property over time so as to
derive a time-dependent measurement profile, b)
5   defining a set of predictor variables which
sufficiently define the data of the time-dependent
profile, c) deriving a model that represents the
relationship between a diagnostic output and the set
of predictor variables, and d) utilizing the model to
10   predict the existence of a congenital or acquired
imbalance or therapeutic condition in the unknown
sample relative to the diagnostic output. In one
embodiment, training data is provided by performing a
plurality of assays on known samples, the model is a
15   multilayer perceptron, the relationship between the
diagnostic output and the set of predictor variables
is determined by at least one algorithm, and the at
least one algorithm is a back propagation learning
algorithm. In a second embodiment of the present
20   invention, the relationship between the diagnostic
output and the set of predictor variables is derived
by a set of statistical equations.

Also in the present invention, a plurality of
time-dependent measurement profiles are derived, which
25   time-dependent measurement profiles can be optical
time-dependent measurement profiles such as ones
provided by a automated analyzer for thrombosis and
hemostasis, where a plurality of optical measurements
are taken over time, and where the plurality of
30   optical measurements are normalized. The optical
profiles can include one or more of a PT profile, a
fibrinogen profile, an APTT profile, a TT profile, a
protein C profile, a protein S profile and a plurality
of other assays associated with congenital or acquired
35   imbalances or therapeutic conditions.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a general neuron diagram relating to the embodiment of the present invention utilizing a neural network;

5   Figure 2 is a diagram of a multilayer perceptron for predicting congenital or acquired imbalances or therapeutic conditions, relating to the neural network embodiment of the present invention;

Figure 3 is an optical profile with first and
10   second derivatives of a normal clotting sample;

Figure 4 is an illustration of two learning curves;

Figure 5 is an illustration of an unstable learning curve;

15   Figure 6 is a graph showing a comparison of training and cross-validation learning curves;

Figure 7 is a graph showing a comparison of training error for training tolerances of 0.0 and 0.1;

Figure 8 is a ROC illustrating the effect of
20   decision boundary on classification;

Figure 9 is a Table comparing hidden layer size with prediction error;

Figure 10 is a receiver operator characteristic plot related to predicting an abnormality in relation
25   to Factor VIII;

Figure 11 is a graph demonstrating the ability to predict actual Factor VIII activity;

Figure 12 is a receiver operator characteristic plot related to predicting an abnormality in relation
30   to Factor X; and

-8-

Figure 13 is a chart listing examples of predictor variables for use in the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

5   In the present invention, both a method and apparatus are provided for predicting the presence of at least one congenital or acquired imbalance or therapeutic condition. As one of the first steps of the method, one or more time-dependent measurements

10   are performed on an unknown sample. The term "time-dependent measurement" is referred to herein to include measurements derived from assays (e.g. PT, APTT, fibrinogen, protein C, protein S, TT, ATIII, plasminogen and factor assays). The terms "unknown

15   sample" and "clinical sample" refer to a sample, such as one from a medical patient, where a congenital or acquired imbalance or therapeutic condition associated with thrombosis/hemostasis is not known (or, if suspected, has not been confirmed). In the present

20   invention, a coagulation property is measured over time so as to derive a time-dependent measurement profile. In a preferred embodiment, the time-dependent measurement is an optical measurement for deriving an optical profile. For example, a PT

25   profile, a fibrinogen profile, a TT profile, an APTT profile and/or variations thereof can be provided where, an unknown sample is analyzed for clot formation based on light transmittance over time through the unknown sample. In another preferred

30   embodiment, two (or more) optical profiles are provided, such as both a PT profile and an APTT profile.

       After the time-dependent measurement profiles are provided, a set of predictor variables are defined

-9-

which sufficiently define the data of the time-
dependent profile. One or more predictor variables
comprise the set. And, in one embodiment, three or
more, and in a preferred embodiment, four or more

5   predictor variables were found to desirably make up
the set. It was found that the characteristics of the
time-dependent measurement profile could best be
defined by one or more predictor variables, including
the minimum of the first derivative of the optical

10   profile, the time index of this minimum, the minimum
of the second derivative of the optical profile, the
time index of this minimum, the maximum of the second
derivative, the time index of this maximum, the
overall change in transmittance during the time-

15   dependent measurement, clotting time, slope of the
optical profile prior to clot formation, and slope of
the optical profile after clot formation.

After defining the set of predictor variables, a
model is derived which represents the relationship

20   between a congenital or acquired imbalance or
therapeutic condition and the set of predictor
variables. This model can be derived from a neural
network in one embodiment of the present invention.
In another embodiment, the model is derived via a set

25   of statistical equations.

Neural networks represent a branch of artificial
intelligence that can be used to learn and model
complex, unknown systems given some known data from
which it can train. Among the features of neural

30   networks that make them an attractive alternative for
modeling complex systems are :

1.   They can handle noisy data well and recognize
      patterns even when some of the input data are
      obscured or missing.

-10-

2.   It is unnecessary to determine what factors are
        relevant <u>a priori</u>, since the network will
        determine during the training phase what data are
        relevant, assuming there are at least some
5   meaningful parameters in the set.

        Neural networks are formed from multiple layers
of interconnected neurons like that shown in Figure 1.
Each neuron has one output and receives input $i_1...i_n$
from multiple other neurons over connecting links, or
10   *synapses*. Each synapse is associated with a synaptic
weight, w_j. An adder Σ or linear combiner sums the
products of the input signals and synaptic weights
i_j*w_j. The linear combiner output sum_1 and θ_1 (a
threshold which lowers or a bias which raises the
15   output) are the input to the activation function $f()$.
The synaptic weights are learned by adjusting their
values through a learning algorithm.

        After deriving the model, whether based on neural
networks or statistical equations, the model is
20   utilized to predict the existence of a congenital or
acquired imbalance or therapeutic condition in the
unknown sample relative to the time-dependent
measurement profile(s). As such, a congenital or
acquired imbalance or therapeutic condition can be
25   predicted. Conditions which can be predicted as being
abnormal in the present invention can include, among
others, a) factor deficiencies, e.g. fibrinogen,
Factors II, V, VII, VIII, IX, X, XI and XII, as well
as ATIII, plasminogen, protein C, protein S, etc., b)
30   therapeutic conditions, e.g. heparin, coumadin, etc.,
and c) conditions such as lupus anticoagulant. In one
embodiment of the present invention, the method is
performed on an automated analyzer. The time-
dependent measurement profile, such as an optical data
35   profile, can be provided automatically by the

SUBSTITUTE SHEET (RULE 26)

-11-

automated analyzer, where the unknown sample is
automatically removed by an automated probe from a
sample container to a test well, one or more reagents
are automatically added to the test well so as to
5   initiate the reaction within the sample. A property
over time is automatically optically monitored so as
to derive the optical profile. The predicted
congenital or therapeutic condition can be
automatically stored in a memory of an automated
10   analyzer and/or displayed on the automated analyzer,
such as on a computer monitor, or printed out on
paper. As a further feature of the invention, if the
predicted congenital or acquired imbalance or
therapeutic condition is an abnormal condition, then
15   one or more assays for confirming the existence of the
abnormal condition are performed on the automated
analyzer. In fact, in a preferred embodiment, the one
or more confirming assays are automatically ordered
and performed on the analyzer once the predicted
20   condition is determined, with the results of the one
or more confirming assays being stored in a memory of
the automated analyzer and/or displayed on the
analyzer.

25   EXAMPLE 1:   Prediction of Heparin in Sample

This example shows a set of predictor variables
that adequately describe screening assay optical
profiles, develops an optimal neural network design,
and determines the predictive capabilities of an
30   abnormal condition associated with
thrombosis/hemostasis (in this case for the detection
of heparin) with a substantial and well-quantified
test data set.

-12-

Simplastin™ L, Platelin™ L, calcium chloride
solution (0.025 M), imidazole buffer were obtained
from Organon Teknika Corporation, Durham, NC, 27712,
USA. All plasma specimens were collected in 3.2% or
5   3.8% sodium citrate in the ratio of one part
anticoagulant to nine parts whole blood. The tubes
were centrifuged at 2000 g for 30 minutes and then
decanted into polypropylene tubes and stored at -80°C
until evaluated. 757 specimens were prepared from 200
10   samples. These specimens were tested by the following
specific assays: FII, FV, FVII, FVIII, FIX, FX, FXI,
FXII, heparin, fibrinogen, plasminogen, protein C,
and AT-III. Samples represented normal patients, a
variety of deficiencies, and therapeutic conditions.
15   Of the specimen population 216 were positive for
heparin determined by a heparin concentration greater
than 0.05 units/ml measured with a chromogenic assay
specific for heparin. The remaining specimens,
classified as heparin-negative, included normal
20   specimens, a variety of single or multiple factor
deficiencies, and patients receiving other therapeutic
drugs. Positive heparin samples ranged to 0.54
units/ml.

PT and APTT screening assays were performed on
25   each specimen utilizing two automated analyzers (MDA™
180s) and multiple reagent and plasma vials (Organon
Teknika Corporation, Durham NC 27712, USA) over a
period of five days. When clot-based coagulation
assays are performed by an automated optically-based
30   analyzer such as the MDA 180, data are collected over
time that represents the normalized level of light
transmission through a sample as a clot forms (the
optical profile). As the fibrin clot forms, the
transmission of light is decreased. The optical
35   profile was stored from each test.

-13-

The network configuration chosen, a multilayer
perceptron (MLP) maps input predictor variables from
the PT and APTT screening assays to one output
variable (see Figure 2) which represents a single
5   specified condition. A similar network was also
employed for PT-only variables and APTT-only
variables. This specific MLP consists of three
layers: the input layer, one hidden layer, and the
output layer.

10   A normal optical profile is shown in Figure 3.
The set of predictor variables were chosen with the
intent of describing optical profiles as completely as
possible with a minimum number of variables. They are
summarized in Table 1 where t is time from initiation
15   of reaction, T is normalized light transmission
through the reaction mixture, and pv_{jk} is the kth
predictor variable of assay j.

The predictor variables were scaled to values
20   between 0 and 1, based on the range of values observed
for each variable for assay type k

$$i_j = f\left(pv_{jk}, \left(pv_{j\_n,k}\right)_{\min}, \left(pv_{j\_n,k}\right)_{\max}\right).$$

25   The input variable set includes $i_{1...7}$ for both a PT
assay and APTT assay for each specimen. For known
output variable values,   heparin samples with
results of greater than 0.05 units/ml were
considered positive and assigned a value of 1 while
30   negative   samples were assigned a value of 0.

As the ratio of training set sample to
the number of weights in a network decreases, the

-14-

probability of generalizing decreases, reducing the
confidence that the network will lead to correct
classification of future samples taken from the same
distribution as the training set. Thus, small samples

5   sizes, then can lead to artificially high
classification rates. This phenomenon is known as
overtraining. In order to achieve a true accuracy
rate of 80%, a guideline for the number of samples in
the training set is approximately five times the

10   number of weights in the network. For most of this
work, a 14-6-1 network was used, leading to an upward
bound on the sample size of $O(450)$. To monitor and
evaluate the performance of the network and its
ability to generalize, a cross-validation set is

15   processed at the end of each training epoch. This
cross-validation set is a randomly determined subset
of the known test set that is excluded from the
training set.

Once the input predictor variables and output

20   values were determined for all specimen optical
profiles, the 757 sets of data were randomly
distributed into two groups: 387 were used in the
training set and 370 were used in the cross-validation
set. These same two randomly determined sets were

25   used throughout all the experiments.

All synaptic weights and threshold values were
initialized at the beginning of each training session
to small random numbers.

The error-correction learning rule is an

30   iterative process used to update the synaptic weights
by a method of gradient descent in which the network
minimizes the error as pattern associations (known
input-output pairs) in the training set are presented
to the network. Each cycle through the training set

35   is known as an epoch. The order or presentation of
the pattern associations was the same for all epochs.

-15-

The learning algorithm consists of six steps which
make up the forward pass and the backward pass. In
the forward pass, the hidden layer neuron activations
are first determined

5

$$h = F(iW1 + \theta_h)$$

where **h** is the vector of hidden-layer neurons, **i** the
vector of input-layer neurons, W1 the weight matrix
10   between the input and hidden layers, and $F()$ the
activation function. A logistic function is used as
the activation function

$$F(x) = \frac{1}{1 + e^{-x}}.$$

15

Then the output-layer neurons are computed

$$o = F(hW2 + \theta_o)$$

20   where **o** represents the output layer, **h** the hidden
layer and W2 the matrix of synapses connecting the
hidden layer and output layers. The backward pass
begins with the computation of the output-layer error

25    $$e_0 = (o - d),$$

where **d** is the desired output. If each element of e_o
is less than some predefined training error tolerance
vector TE_{tol}, than the weights are not updated during
30   that pass and the process continues with the next
pattern association. A training error tolerance of

-16-

0.1 was used in all experiments unless otherwise specified. Otherwise, the local gradient at the output layer is then computed:

5
$$g_o = o(1 - o)e_0.$$

Next, the hidden-layer local gradient is computed:

$$g_h = h(1 - h)W2g_o.$$

10

Once the hidden layer error is calculated, the second layer of weights is adjusted

$$W2_m = W2_{m-1} + \Delta W2$$

15

where

$$\Delta W2 = \eta hg_o + \gamma \Delta W2_{m-1}.$$

20   is the learning rate, γ is the momentum factor, and m is the learning iteration. The first layer of weights is adjusted in a similar manner

$$W1_m = W1_{m-1} + \Delta W1$$

25

where

$$\Delta W1 = \eta ie + \gamma \Delta W1_{m-1}.$$

-17-

The forward pass and backward pass are repeated for all of the pattern associations in the training set, referred to as an epoch, 1000 times . At the end of each epoch, the trained network is applied to the

5   cross-validation set.

Several methods were employed to measure the performance of the network's training. Error, E, for each input set was defined as

10

$$E = \sqrt{\frac{1}{N}\sum_{q=1}^{N}\left(d_q - o_q\right)^2} .$$

The learning curve is defined as the plot of E versus epoch. The percent classification, φ, describes the

15   percent of the total test set (training and cross-validation) that is correctly classified based on some defined decision boundary, β. Receiver-Operating Characteristic (ROC) plots have also been utilized to describe trained networks' ability to discriminate

20   between the alternative possible outcome states. In these plots, measures of sensitivity and specificity are shown for a complete range of decision boundaries. The sensitivity, or true-positive fraction is defined as

25

$$sensitivity = \frac{true\ positive}{true\ positive + false\ negative}$$

and the false-positive fraction , or (1-specificity) is defined as

30

$$(1 - specificity) = \frac{false\ positive}{false\ positive + true\ negative}$$

These ROC plots represent a common tool for evaluating clinical laboratory test performance.

Using the test set described, experiments were performed to determine if the presence of heparin could be predicted with this method. First, experiments were conducted to determine optimal error-correction backpropagation learning parameters: (1) hidden layer size, (2) learning rate, and (3) momentum. Additional experiments were also conducted to compare the performance of networks based on PT and APTT assays alone with that of one combining the results of both, the effect of the training error tolerance, and the decision boundary selection.

Figure 9 shows the effect of the hidden layer size on the training and cross validation error and the percent correct classification for the optimal decision boundary, defined as the decision boundary which yielded the lowest total number of false positives and false negatives from the total test set. As the hidden layer size is increased, the error is decreased. However, the ability to generalize does not increase after a hidden layer size of 6. The most significant benefit in terms of both error and percentage correct classification is between 4 and 6. A hidden layer size of 6 was used for the remainder of the experiments.

A series of experiments were conducted with $\eta = \{0.01, 0.1, 0.5, 0.9\}$ and $\gamma = \{0.0, 0.1, 0.5, 0.9\}$. Figure 4 shows the learning curves for two of the best combinations of parameters. Figure 5 shows an example learning curve

-19-

when the learning rate is so high it leads to
oscillations and convergence to a higher E.   In
general, as $\eta \rightarrow 0$ the network converged to a lower E

and as $\gamma \rightarrow 1$, the rate of convergence improved.   As

5    $\eta \rightarrow 1$, the value of E converged too increased and

oscillations increased.   In addition, as $\eta \rightarrow 1$, $\gamma \rightarrow 1$
exacerbated the oscillations.

     Figure 6 shows a comparison of the learning curve
10   for the training set and cross-validation set for
     $\eta=0.5$ and $\gamma=0.1$.   It is a primary concern when
     developing neural networks, and it has been previously
     shown that it is important to look not only at the
     error in the training set for each cycle, but also the
15   cross-validation error.
     Figure 7 shows the learning curve $\eta=0.5$ and $\gamma=0.I$
     and a learning tolerance of 0.0 and 0.1.   These
     results suggest that a small learning tends to
     smoothen the convergence of the learning process.
20   Figure 8 shows the ROC plot for networks trained
     with the predictor variables from each of the two
     screening assays with that of them combined.   In the
     single assay cases, the hidden layer size was 3.
     While using the data from one assay does lead to some
25   success, using the information from both assays makes
     a significant improvement in the ability of the
     network to correctly predict the presence of heparin.
     This graph indicates that a 90% true positive
     proportion can be achieved with a false positive
30   proportion of 15%.   Using a single assay, a 60-70%
     true positive proportion can be achieved with a false
     positive proportion of approximately 15%.

-20-

EXAMPLE 2: Factor VIII

Similar tests were run as in Example 1. As can
be seen in Figures 10 and 11, two training sessions
were conducted for predicting a Factor VIII condition
5   in an unknown sample. Figure 10 is a receiver
operator characteristic plot related to predicting an
abnormality in relation to Factor VIII. In Figure 10,
everything below 30% activity was indicated as
positive, and everything above 30% was indicated as
10   negative. Cutoff values other than 30% could also be
used. In this Example, the activity percentage has a
known accuracy of approximately + or - 10%. In Figure
11, the actual percent activity was utilized as the
output.

15

EXAMPLE 3: Factor X

As can be seen in Figure 12, the method of the
present invention was run similar to that as in
Example 2, where here an abnormality in Factor X
20   concentration was predicted from unknown samples.
Everything below 30% activity was indicated as
positive, and everything above 30% was indicated as
negative. Cutoff values other than 30% could also be
used.

25   The results of the cross-validation sample sets
throughout the experiments indicate that the sample
size was sufficient for the network to generalize.
While the random distribution of the training and
cross-validation sets were held constant throughout
30   the experiments presented, other distributions have
been used. These distributions, while all yielding
different results, still lead to the same general
conclusion.

Many alternatives for or additions to the set of
35   predictor variables were explored. This included
coefficients of a curve fitted to the data profile,

-21-

pattern recognition, and clot time-based parameters.
Low order functions tend to lose information due to
their poor fit, and high order functions tend to lose
information in their multiple close solutions.   Clot-
5   based parameters, such as clot time, slope in the
section prior to the initiation of clot formation, and
afterwards, are often available, but not always
(because in some samples, the clot time is not
detectable).   The successful results observed
10   indicate that the set of predictor variables used are
effective for predicting congenital or acquired
imbalances or therapeutic conditions.

The optimization of the network learning
algorithm's parameters made significant differences in
15   its performance.   In general, performance was best
with low learning rates, high momentum rates, some
small training error tolerance, and a hidden layer
size approximately half of the size of the input
layer.

20   It is to be understood that the invention
described and illustrated herein is to be taken as a
preferred example of the same, and that various
changes in the method and apparatus of the invention
may be resorted to, without departing from the spirit
25   of the invention or scope of the claims.

-22-

WE CLAIM:

1.   A method for predicting the presence of at least
one congenital or acquired imbalance or therapeutic
5   condition associated with thrombosis/hemostasis from
at least one time-dependent measurement profile,
comprising:

     a)   performing at least one time-dependent
measurement on an unknown sample and measuring a
10   respective property over time so as to derive a time-
dependent measurement profile;

     b)   defining a set of a plurality of predictor
variables which sufficiently define the data of the
time-dependent measurement profile;

15   c)   deriving a model that represents the
relationship between the congenital or acquired
imbalance or therapeutic condition, and the set of
predictor variables; and

     d)   utilizing the model of step c) to predict the
20   existence of the congenital or acquired imbalance or
therapeutic condition in the unknown sample.

     2.   A method according to claim 1, wherein said
at least one time-dependent measurement profile is at
25   least one optical profile.

     3.   A method according to claim 2, wherein said
at least one optical profile is provided by an
automated analyzer for thrombosis and hemostasis
30   testing.

     4.   A method according to claim 2, wherein a
plurality of optical measurements at one or more
wavelengths are taken over time so as to derive said
35   at least one optical profile, said optical

-23-

measurements corresponding to changes in light
scattering and/or light absorption in the unknown
sample.

5   5.   A method according to claim 2, wherein a
plurality of optical measurements are taken over time
so as to derive said at least one optical profile, and
wherein said plurality of optical measurements are
each normalized to a first optical measurement.

10

          6.   A method according to claim 3, wherein in
step a) said at least one optical profile is provided
automatically by said analyzer, whereby said unknown
sample is automatically removed by an automated probe

15   from a sample container to a test well, one or more
reagents are automatically added to said test well so
as to initiate said property changes within said
sample, and the development of said property over time
is automatically optically monitored so as to derive

20   said optical data profile.

          7.   A method according to claim 6, wherein after
step d), a predicted congenital or acquired imbalance
or therapeutic condition is automatically stored in a

25   memory of said automated analyzer and/or displayed on
said automated analyzer.

          8.   A method according to claim 6, wherein in
step d), one or more assays for confirming the

30   existence of said congenital or acquired imbalance or
therapeutic condition is automatically performed.

          9.   A method according to claim 8, wherein said
one or more confirming assays are automatically

35   ordered and performed on said analyzer, with results

-24-

of said one or more assays being stored in a memory of said automated analyzer and/or displayed on said analyzer.

5   10.   A method according to claim 1, further comprising:

before step a), providing a set of data from known samples, which data is used in step c) for deriving said model.

10

      11.   A method according to claim 10, wherein said data from known samples is provided by performing a plurality of assays on said known samples.

15   12.   A method according to claim 10, wherein said model of step c) is a neural network.

      13.   A method according to claim 1, wherein said relationship in step c) is determined via at least one
20   automated algorithm.

      14.   A method according to claim 1, wherein in step a), a plurality of time-dependent measurement profiles are derived for use in step b).

25

      15.   A method according to claim 14, wherein said plurality of time dependent measurement profiles includes at least two profiles from assays initiated with PT reagents, APTT reagents, fibrinogen reagents
30   and TT reagents.

      16.   A method according to claim 13, wherein said model is a multilayer perceptron, and wherein said at

-25-

least one algorithm is a back propagation learning
algorithm.

17.   A method according to claim 1, wherein said
5   set of predictor variables includes a plurality of: a
minimum of the first derivative of the profile, a time
index of the minimum of the first derivative, a
minimum of the second derivative of the profile, a
time index of the minimum of the second derivative, a
10   maximum of the second derivative of the profile, a
time index of the maximum of the second derivative, an
overall change in the coagulation parameter during the
time-dependent measurement on the unknown sample, a
clotting time, a slope of the profile prior to clot
15   formation, and a slope of the profile after clot
formation.

18.   A method according to claim 17, wherein
three or more of said predictor variables are within
20   said set.

19.   A method according to claim 18, wherein more
than three of said predictor variables are within said
set.
25

20.   A method according to claim 1, wherein said
unknown sample is a sample from a medical patient, and
wherein in step d), both said model and additional
patient medical data are utilized for predicting the
30   existence of said congenital or acquired imbalance or
therapeutic condition.

21.   An apparatus for performing at least one
time-dependent measurement on an unknown sample to
35   derive at least one time-dependent measurement

-26-

profile, and predicting the presence of at least one
congenital or acquired imbalance or therapeutic
condition associated with thrombosis/hemostasis from
the at least one time-dependent measurement profile,
5   comprising:

       means for performing at least one time-dependent
measurement on an unknown sample and measuring a
respective property over time so as to derive a time-
dependent measurement profile;

10   means for defining a set of a plurality of
predictor variables which sufficiently define the data
of the time-dependent measurement profile;

       means for deriving a model that represents the
relationship between the congenital or acquired
15   imbalance or therapeutic condition, and the set of
predictor variables; and

       means for utilizing the model of step c) to
predict the existence of the congenital or acquired
imbalance or therapeutic condition in the unknown
20   sample.

       22.   An apparatus according to claim 21, wherein
said means for performing at least one time-dependent
measurement comprises an optical system for performing
25   at least one optical measurement over time and so as
to derive an at least one optical profile.

       23.   An apparatus according to claim 22, wherein
said optical system is part of an automated analyzer
30   for thrombosis and hemostasis testing.

       24.   An apparatus according to claim 22, wherein
said optical means comprises a means for performing a
plurality of optical measurements at one or more
35   wavelengths over time so as to derive said at least

-27-

one optical profile, said optical measurements
corresponding to changes in light scattering and/or
light absorption in the unknown sample.

5   25.   An apparatus according to claim 22, wherein
in said optical system, a plurality of optical
measurements are taken over time so as to derive said
at least one optical profile, and wherein said
plurality of optical measurements are each normalized
10   to a first optical measurement.

       26.   An apparatus according to claim 23 which is
an automated analyzer for thrombosis and hemostasis
testing, and wherein said at least one optical profile
15   is provided automatically by said analyzer, whereby
said unknown sample is automatically removed by an
automated probe from a sample container to a test
well, one or more reagents are automatically added to
said test well so as to initiate said property changes
20   within said sample, and the development of said
property over time is automatically optically
monitored so as to derive said optical data profile.

       27.   An apparatus according to claim 26, further
25   comprising at least one of a memory and a display
wherein a predicted congenital or acquired imbalance
or therapeutic condition is automatically stored in
said memory of said automated analyzer and/or
displayed on said display of said automated analyzer.
30

       28.   An apparatus according to claim 26, further
comprising means for automatically performing one or
more assays for confirming the existence of said
congenital or acquired imbalance or therapeutic
35   condition.

-28-

29. An apparatus according to claim 28, wherein said means for performing one or more confirming assays is an automatic performing means wherein said
5 confirming assays are automatically ordered and performed on said analyzer, with results of said one or more assays being stored in a memory of said automated analyzer and/or displayed on a display of said analyzer.
10

30. An apparatus according to claim 21, further comprising means for providing a set of data from known samples, which data is used in step c) for deriving said model.
15

31. An apparatus according to claim 30, wherein said data from known samples is provided by said means for performing a plurality of assays on said known samples.
20

32. An apparatus according to claim 30, wherein said means for deriving a model is a means for deriving a model by means of a neural network.

25 33. An apparatus according to claim 21, wherein said relationship determined by said deriving means comprises a means for determining said relationship via at least one automated algorithm.

30 34. An apparatus according to claim 21, wherein said means for performing at least one time-dependent measurement is capable of performing a plurality of time-dependent measurement profiles.

-29-

35.   An apparatus according to claim 34, wherein
said means for performing a plurality of time
dependent measurement profiles includes a means for
performing at least two profiles from assays initiated
5   with PT reagents, APTT reagents, fibrinogen reagents
and TT reagents.

36.   An apparatus according to claim 33, wherein
said model is a multilayer perceptron, and wherein
10   said at least one algorithm is a back propagation
learning algorithm.

37.   An apparatus according to claim 21, wherein
said set of predictor variables includes a plurality
15   of: a minimum of the first derivative of the profile,
a time index of the minimum of the first derivative, a
minimum of the second derivative of the profile, a
time index of the minimum of the second derivative, a
maximum of the second derivative of the profile, a
20   time index of the maximum of the second derivative, an
overall change in the coagulation parameter during the
time-dependent measurement on the unknown sample, a
clotting time, a slope of the profile prior to clot
formation, and a slope of the profile after clot
25   formation.

38.   An apparatus according to claim 37, wherein
three or more of said predictor variables are within
said set.

30

39.   An apparatus according to claim 38, wherein
more than three of said predictor variables are within
said set.

-30-

40. An apparatus according to claim 21, wherein
said unknown sample is a sample from a medical
patient, and wherein said utilizing means comprising a
means for utilizing both said model and additional
5   patient medical data for predicting the existence of
said congenital or acquired imbalance or therapeutic
condition.

FIG. I
Neuron Diagram

FIG. 2
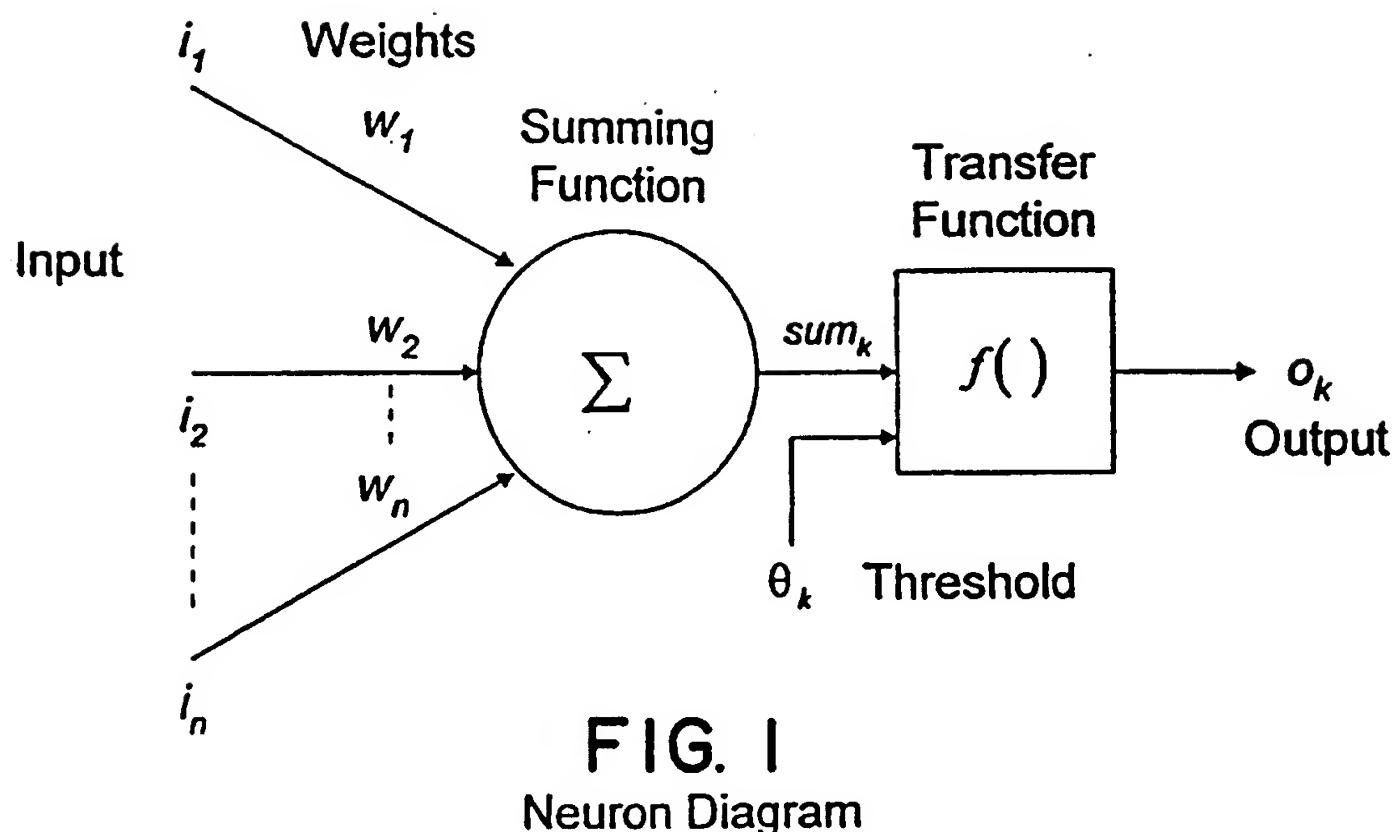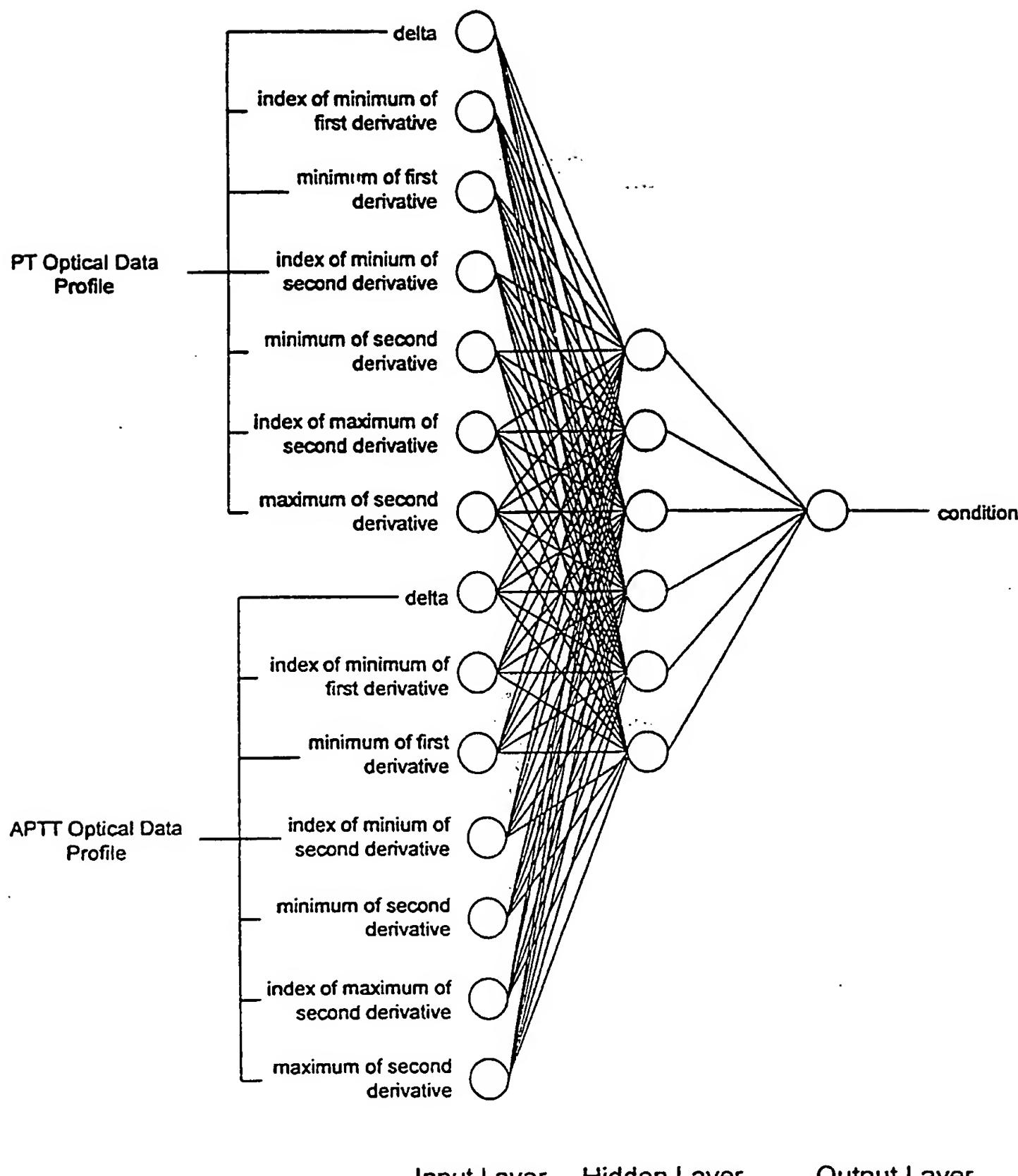
Multilayer Perceptron for Predicting Congenital and Therapeutic Conditions
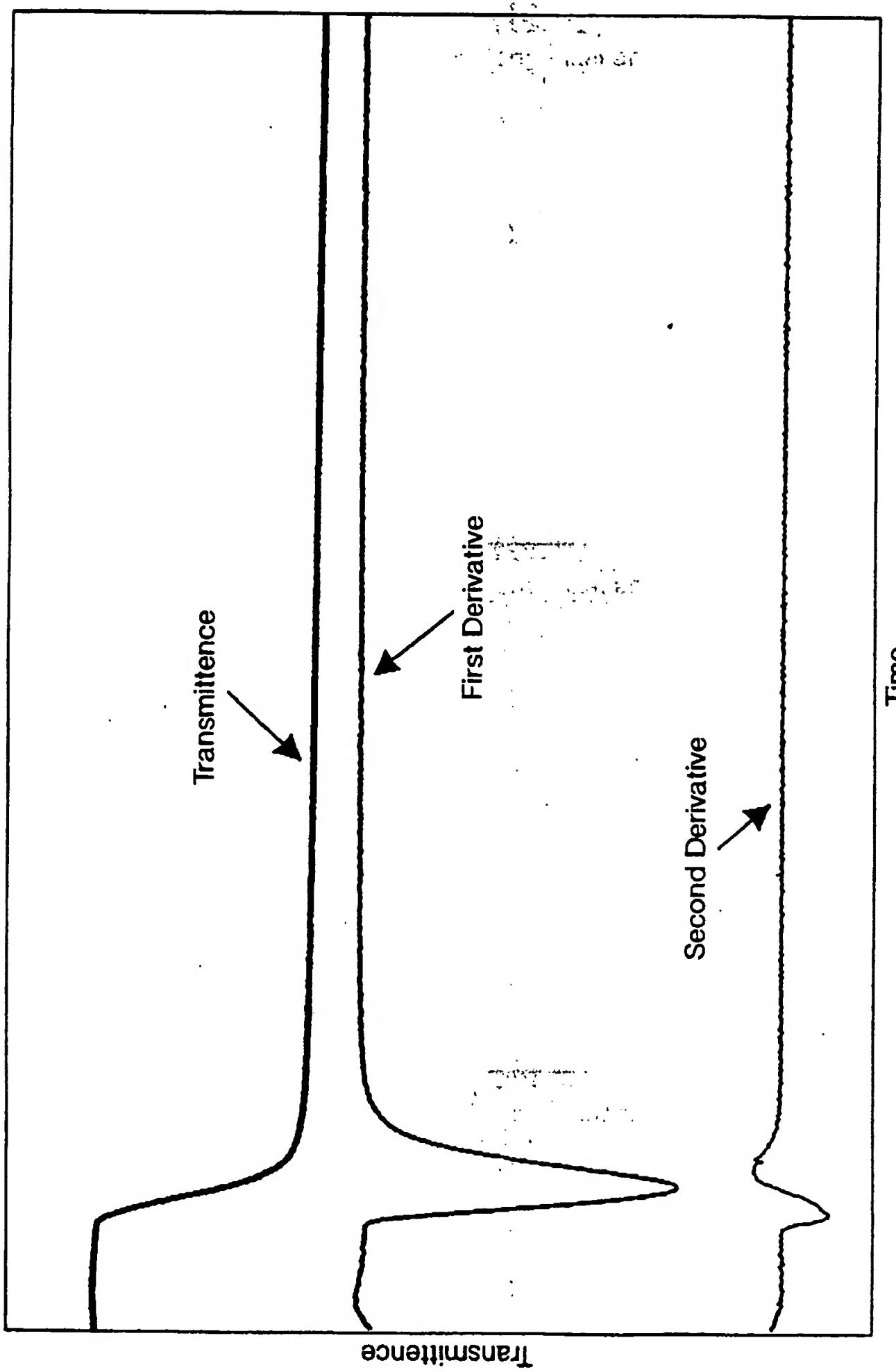
FIG. 3
Normal APTT Optical Profile with First and Second Derivative
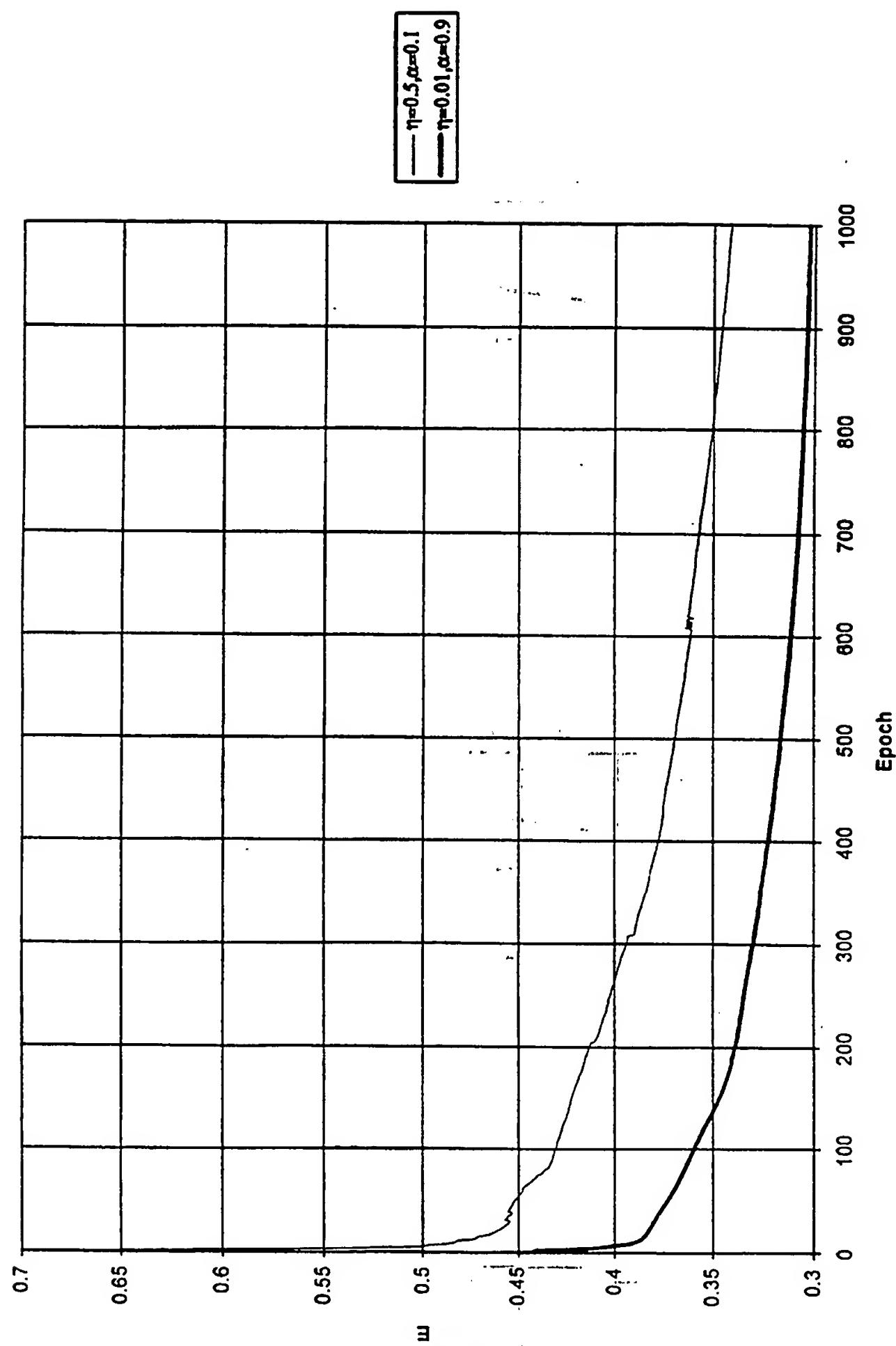
FIG. 4

Two of the Best Learning Curves
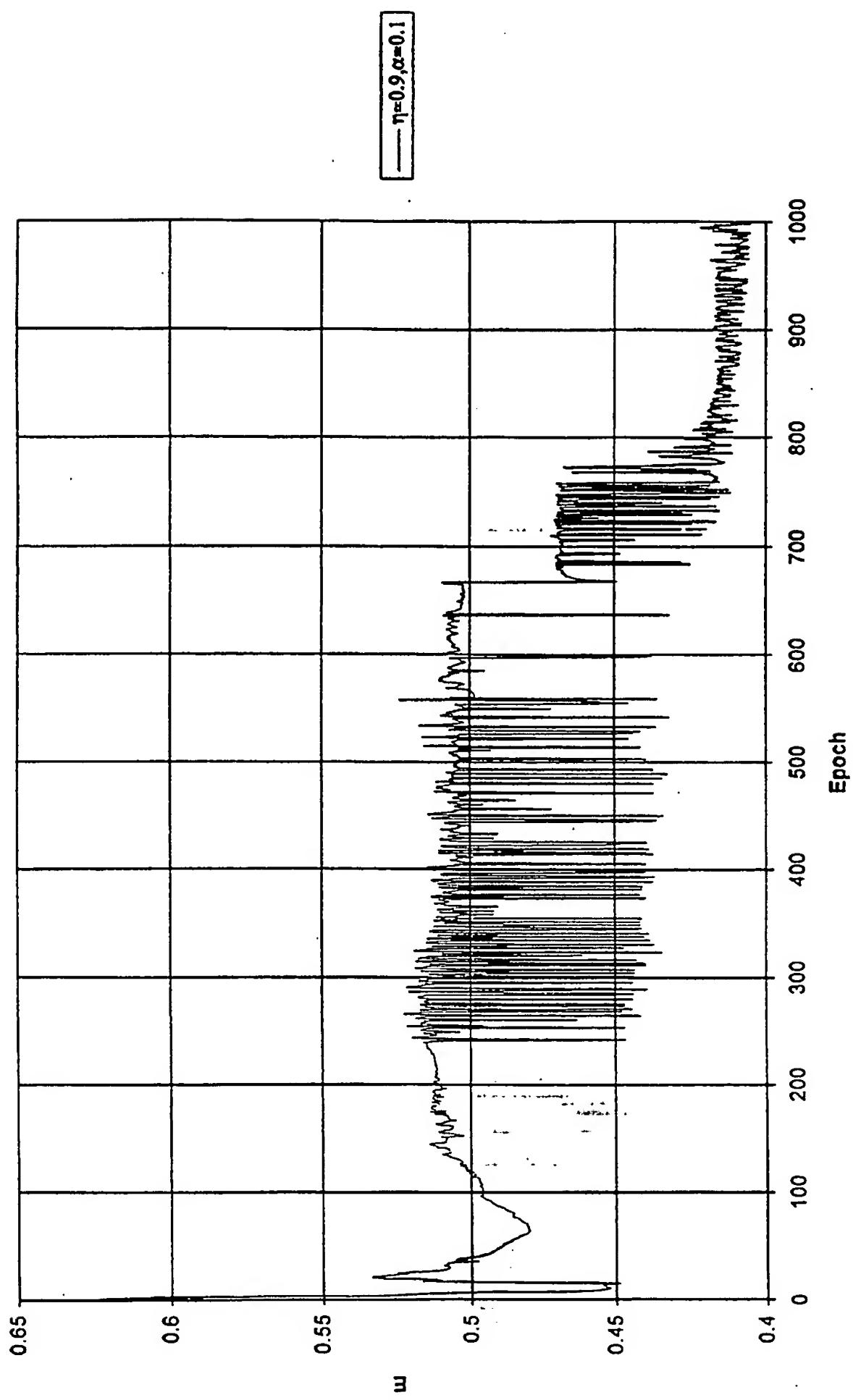
FIG. 5

One of the Most Unstable and Worst Learning Curves
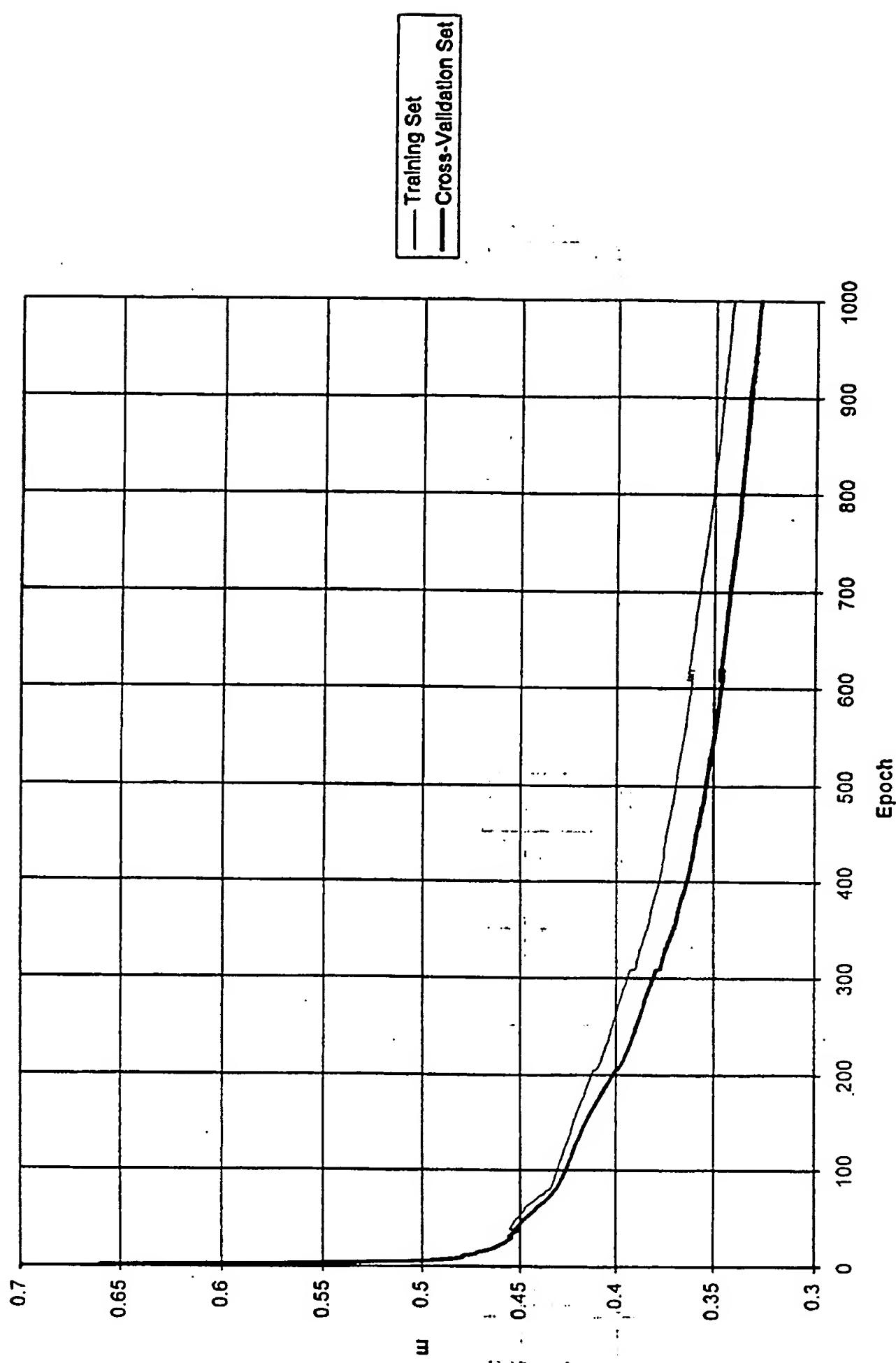
FIG. 6

Comparison of Training and Cross-Validation Learning Curves
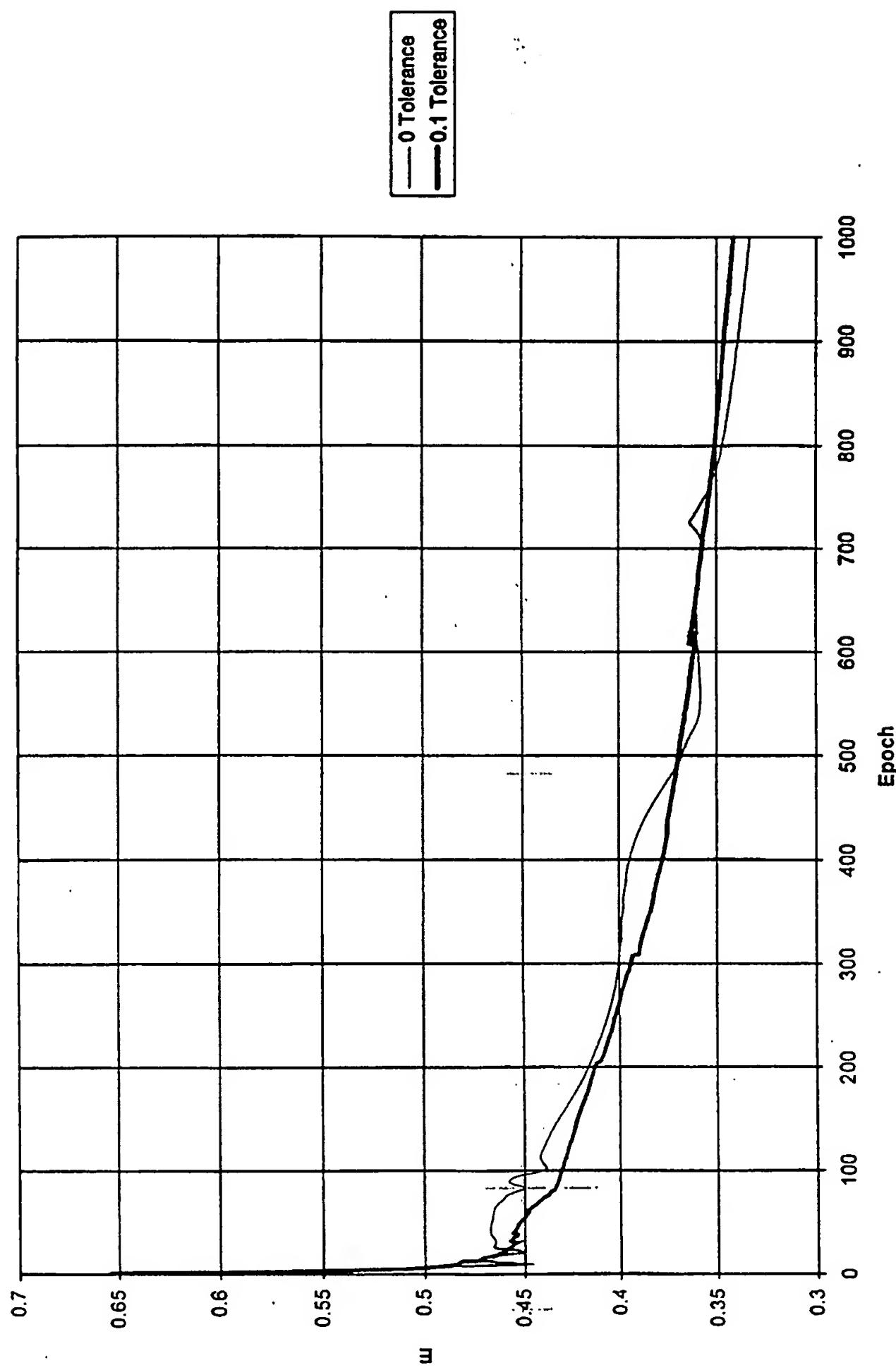
FIG. 7

Comparison of Training Error for Training Tolerances of 0.0 and 0.1
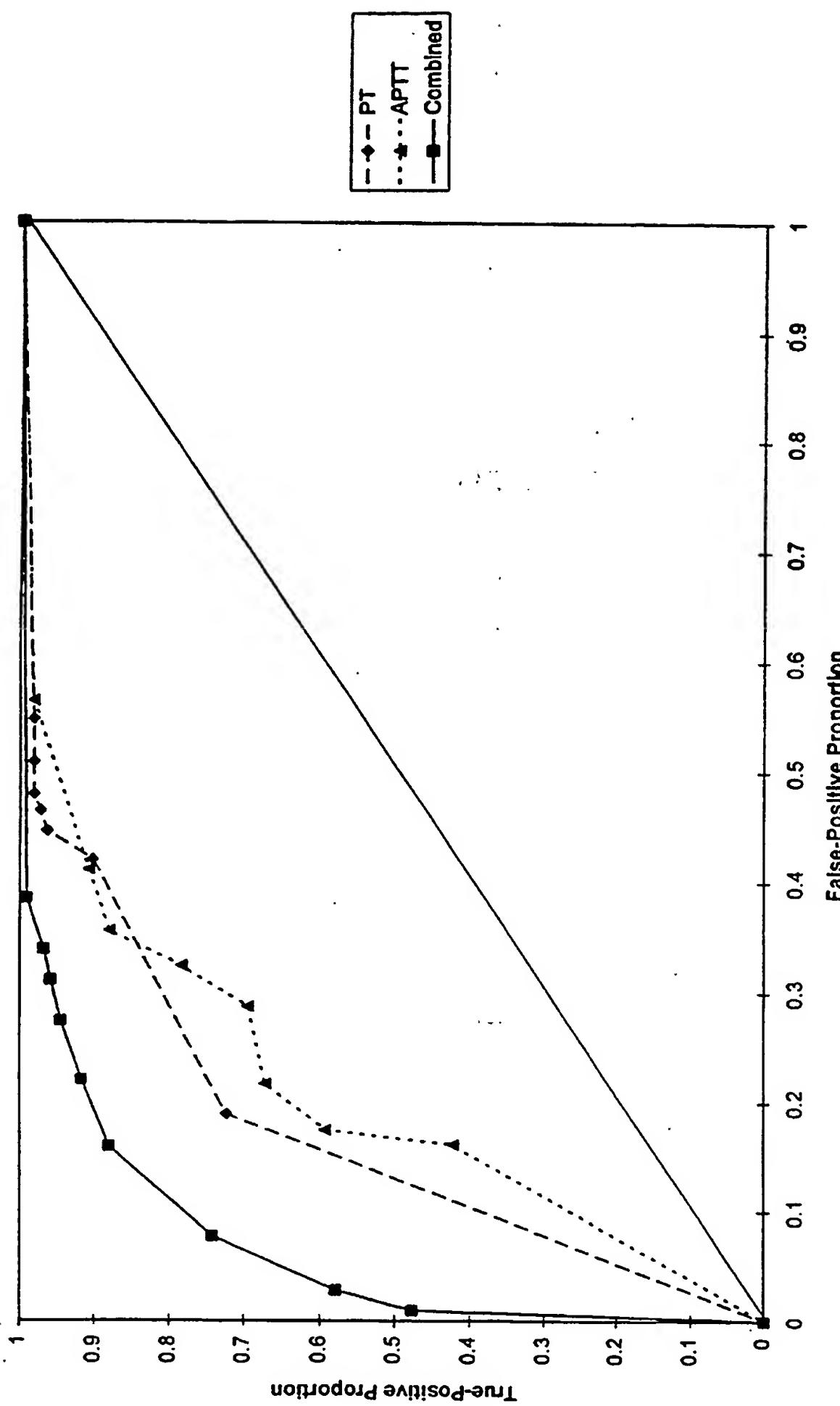
FIG. 8

Effect of Decision Boundary on Classification

Hidden Layer Size	Error		
	E_{tr}	E_{cv}	φODB
2	0.384	0.376	0.848
4	0.386	0.354	0.835
6	0.341	0.328	0.875
8	0.358	0.327	0.857
10	0.346	0.325	0.856
12	0.347	0.322	0.855

FIG. 9

Effects of Various Hidden Layer Sizes on Heparin Network Performance After 1000 Epochs

Receiver Operator Characteristic Plot

Positive: 24
Negative: 684

Hidden Layer: 6
Training: .5
Momentum: .1
Learning Tolerance: .1

FVIII

False-Positive Proportion

True-Positive Proportion

FIG. 10

FIG. 11

FIG. I2
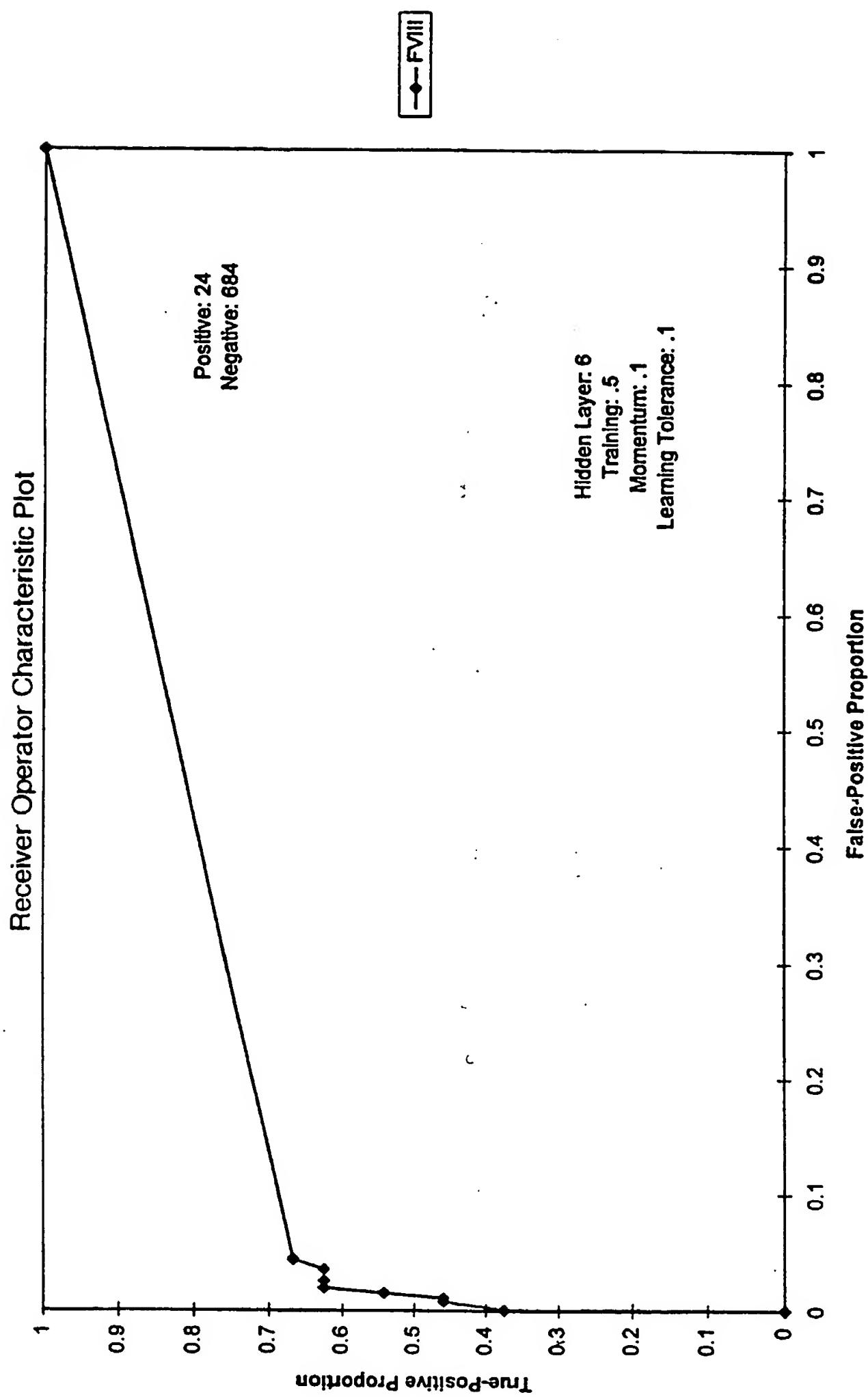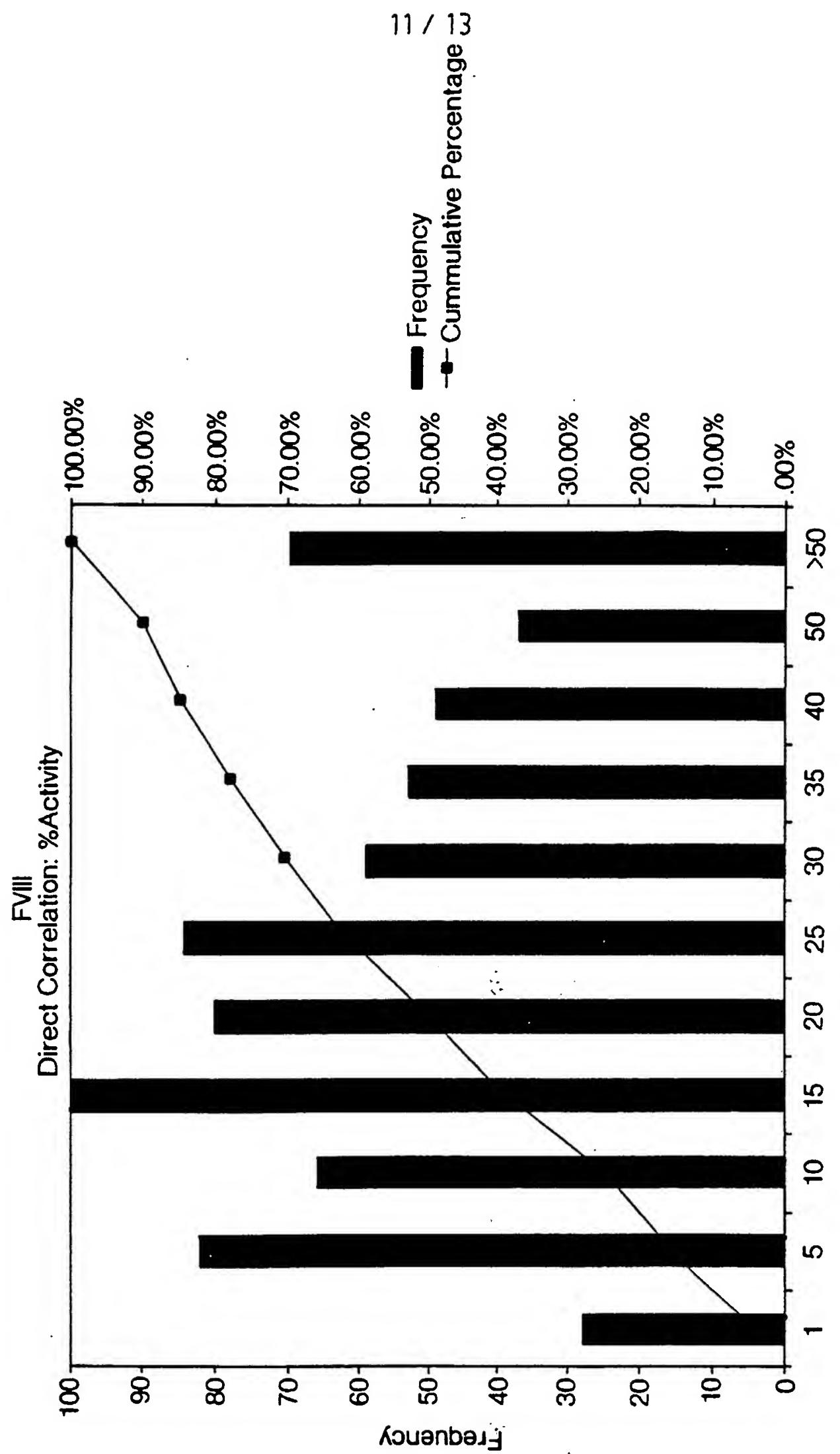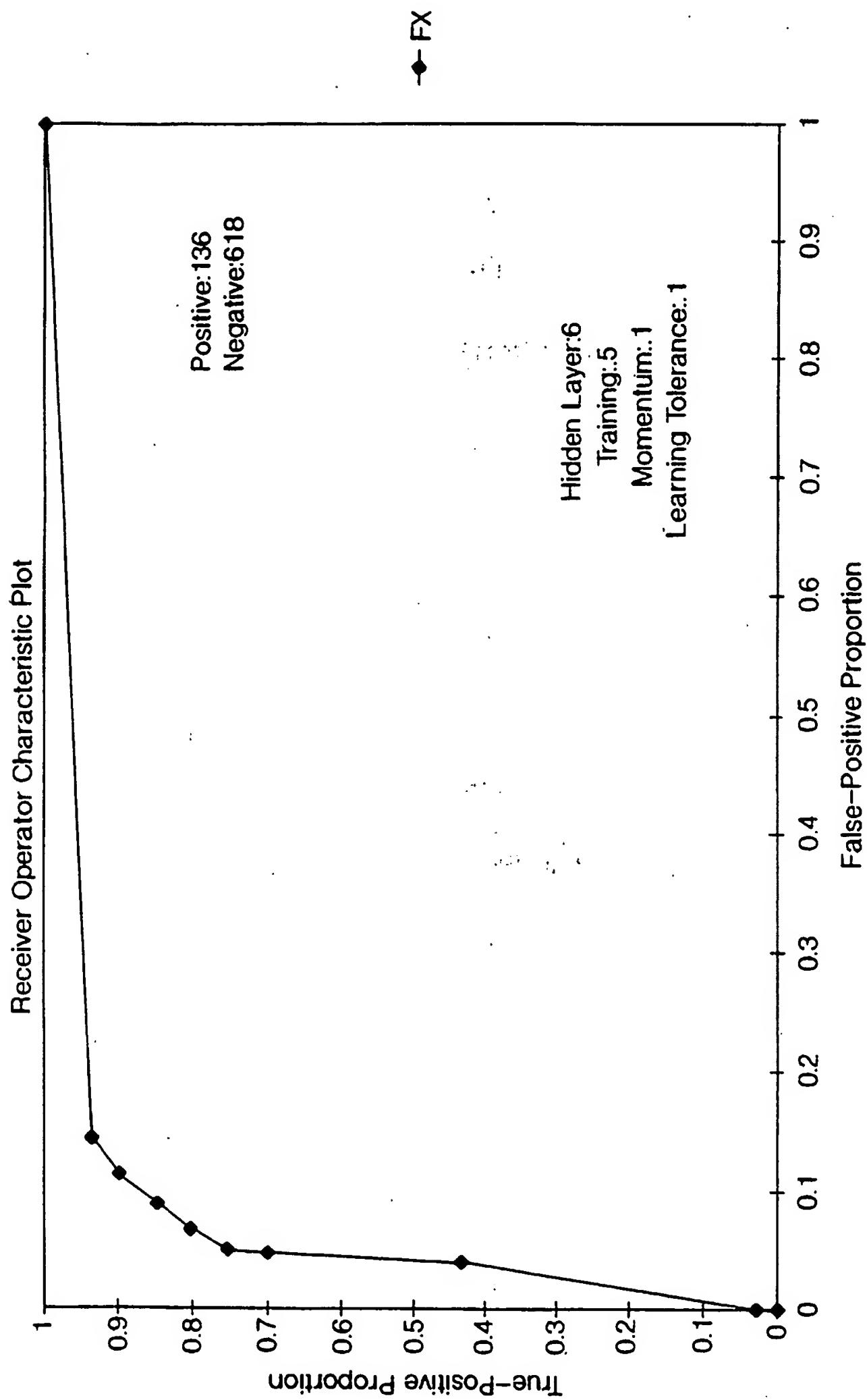
Table 1. Predictor Variables

Predictor Variable	Description
$pv_{f1} = \left(\dfrac{dT}{dt}\right)_{min}$	minimum of the first derivative
$pv_{f2} = t$ at $\left(\dfrac{dT}{dt}\right)_{min}$	time index of the minimum of the first derivative
$pv_{f3} = \left(\dfrac{d_2T}{dt^2}\right)_{min}$	minimum of the second derivative
$pv_{f4} = t$ at $\left(\dfrac{dT^2}{dt^2}\right)_{min}$	index of the minimum of the second derivative
$pv_{f5} = \left(\dfrac{d^2T}{dt^2}\right)_{max}$	maximum of the second derivative
$pv_{f6} = t$ at $\left(\dfrac{dT^2}{dt^2}\right)_{max}$	index of the maximum of the second derivative
$pv_{f7} = T_{f_0} - T_{f_n}$	overall change in transmittence during the reaction

FIG. 13

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G06F 19/00
US CL :364/496

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 364/496-499, 578, 413.01, 413.02, 413.07-413.09; 382/128, 133, 134; 436/50, 63, 69

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 5,156,974 (GROSSMAN ET AL) 20 OCTOBER 1992, see at least the Abstract.	1-40.
A	US, A, 4,998,535 (SELKER ET AL) 12 MARCH 1991, see at least the Abstract.	1-40.
A	US, A, 4,199,748 (BACUS) 22 APRIL 1980, see at least the Abstract.	1-40.

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

Date of the actual completion of the international search	Date of mailing of the international search report
15 AUGUST 1996	09 OCT 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer EMANUEL T. VOELTZ
Facsimile No. (703) 305-3230	Telephone No. (703)305-9714

Form PCT/ISA/210 (second sheet)(July 1992)*